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The role of carbohydrates in wound-healing of sweetpotato roots at low humidity

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ABSTRACT

It has previously been shown that sweetpotato cultivars differ in the efficiency by which their roots can heal wounds at low humidity, although this does not appear to relate to rate of wound-healing at high humidity. It has also been shown previously that there is a negative relationship between cultivar root dry matter (DM) content and efficiency of root wound-healing at low humidity (assessed by a lignification score; LS). Root DM content tends to be negatively related to root sugar levels. The study presented here was undertaken to examine further the role of carbohydrates in root response and ability to heal wounds in the presence of water stress. Data from 17 cultivars confirmed the negative correlation between LS and DM and the positive correlation between LS and root sugar levels. Measurement of sugar levels at the root surface both at the time of wounding and after complete healing (5 days) for 10 cultivars indicated a stronger relationship of LS with final sugar levels than initial DM content. This was confirmed in further experiments using a system of adjacent tissue cuboids cut from the parenchyma which were able to exhibit lignification almost as efficiently as whole roots. With this system it was also possible to demonstrate a relatively rapid accumulation of sugars within 24 h of healing. The data were examined further by the development of linear regression models of LS. Comparison of the levels of variance accounted for by the models indicates that LS is strongly cultivar dependent, and most of the cultivar effect is related to cultivar differences in sugar levels during wound-healing. Differences in sugar levels between roots/cuboids of each cultivar also have an effect. Further, the similarity of the models for the whole roots and cuboids gives us confidence in the validity of using the cuboids to investigate wound-healing of whole roots.

1. Introduction

The short shelf-life of sweetpotato (*Ipomoea batatas*) (an average of 1–2 weeks under marketing conditions with no temperature control) (Rees et al., 2001) is a major constraint both for food security and for marketing of the crop, especially in the developing world where the use of temperature and humidity control is often not feasible. However, sweetpotato germplasm is very diverse, and surveys of sweetpotato cultivars undertaken in East Africa indicate a wide range in shelf-life among cultivars (Rees et al., 2003). The healing response of surface tissues in root crops such as sweetpotato is very important for the protection of damaged roots against water loss and pathogen invasion, and is key to ensuring good keeping quality. It has been demonstrated that the wide range in sweetpotato shelf-life is associated with variability in the efficiency of wound-healing (van Oirschot et al., 2006). Although sweetpotato cultivars are generally all able to heal wounds efficiently when placed at high humidity, they differ significantly in their ability to heal wounds when kept at low humidity (van Oirschot et al., 2000, 2006).

Wound-healing of roots and tubers, notably potatoes, has been studied extensively under controlled high humidity conditions (Walter and Schadel, 1983; Burton, 1989; St Amand and Randle, 1991). However, tissue response at low humidity has been largely overlooked, even though it corresponds more closely to natural conditions and the marketing environment, especially in the absence of temperature/humidity control.

In sweetpotato roots the process of wound-healing involves desiccation of the surface cell layers, followed by lignification of underlying cell layers and finally the formation of a wound periderm by cell division (Artschwager and Starrett, 1931). At low humidity, poor wound-healing is associated with a thick desiccated layer and slow or incomplete lignification (van Oirschot et al., 2006). Continuity of the lignified layer is vital for effective wound-healing, presumably to act as an effective barrier to water loss and pathogen invasion (van Oirschot et al., 2006). A method for assessing efficiency of wound-healing based on assessing the continuity of lignified layers by phloroglucinol staining (lignification score; LS) was developed by van Oirschot et al. (2002, 2006), and has been used as a tool for screening sweetpotato germplasm.

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Comparison of wound-healing at low humidity by sweetpotato roots and potato tubers shows that potato is much more efficient at forming a barrier against water loss (van Oirschot et al., 2006). In potatoes the initial cell wall thickening is in the form of suberin rather than lignin, but, otherwise, the basis for the difference in efficiency is unknown.

A relationship of sweetpotato root dry matter (DM) content both with shelf-life (Rees et al., 2003) and with wound-healing efficiency, has been reported previously (van Oirschot et al., 2002, 2006). Low dry matter content is associated with longer shelf-life and more efficient healing at low humidity. In order to understand the basis for this association, we carried out a screening of a wide range of sweetpotato germplasm originating from many regions of the world (van Oirschot et al., 2002). Cultivars from different regions tended to cluster both by wound-healing efficiency and dry matter content. Thus, African cultivars tended to be both poor healers and have high dry matter content. This led to the view that the association between dry matter content and wound-healing efficiency might not be due to a functional relationship. However, initial experiments reported by van Oirschot et al. (2002), which were designed to allow the characteristics of individual roots rather than cultivars, to be assessed, indicated that the association (low dry matter implies more efficient healing) could be demonstrated even between roots of the same cultivar.

In order to breed sweetpotato cultivars with improved shelf-life it is important to understand the physiological basis for the large differences in wound-healing efficiency. Further, given that high dry matter is an important consumer criterion in Africa, the ideal would be for cultivars that have both high dry matter content and efficient wound-healing at low humidity. This would be possible if there is not a direct functional relationship, and the identification of a few outstanding cultivars with high dry matter and efficient healing supports this possibility.

It has previously been observed that there is no relationship between wound-healing efficiency of a particular cultivar at high humidity and at low humidity (van Oirschot et al., 2002). This indicates that the difference among cultivars is not just in rate of healing, but that the key factor controlling sweetpotato efficiency of wound-healing at low humidity is the tissue response to water stress. Most of the dry matter in sweetpotato roots is in the form of starch, and it is difficult to envisage a mechanism whereby a high concentration of starch grains might interfere directly with wound-healing. However, low dry matter sweetpotato cultivars also tend to have a higher sugar: starch ratio (Rees et al., 2003). This might indicate that the ability to mobilise sugars could be a key factor. In support of this hypothesis, we have previously reported a strong relationship between shelf-life and root monosaccharide levels (Rees et al., 2003), as well as initial experiments indicating a relationship between wound-healing efficiency and sugar levels (van Oirschot et al., 2002). An accumulation of sugars would provide energy for wound-healing and could also be involved in protection against water stress, as conversion of starch into soluble sugars is a mechanism by which plant tissues can increase their osmotic potential. Thus, for example, an increase in sucrose levels on the application of osmotic stress has been observed in leaf discs (Quick et al., 1989) and potato discs (Geigenberger et al., 1997, 1999).

In this report we present data which provide more information on the relationship between sugar content and wound-healing in sweetpotato roots. Specifically we report experiments designed to enable us to look at the sugar levels and their changes near to tissue wounds, during the process of healing at low humidity. We have used a method developed previously (van Oirschot et al., 2006) for assessing wound-healing in terms of a lignification score. We developed a system using small sections of tissue cut from roots that are able to lignify in the same way as wounds in whole roots. We have chosen cultivars with a range of characteristics, in particular including cultivars that deviate from the general relationship between dry matter content and wound-healing efficiency, which should strengthen the rigour of any associations that we find.

2. Materials and methods

2.1. Root supply

Sweetpotatoes (*I. batatas*) were grown in field trials by the International Potato Center (CIP), Nairobi, Kenya. Harvested roots were air-freighted (under temperature controlled conditions to avoid chilling injury) to the NRI, UK.

Apart from the first (Cultivar) experiment, cultivars were chosen to cover a range of dry matter content and wound-healing efficiency as determined in previous experiments (van Oirschot et al., 2002) as follows:

Low DM, good healers: Zapallo, Yanshu, Beauregard.
High DM, good healers: Sinia, Naveto, Cemsa 74–228.
Low DM, poor healers: Hernandez, KSP20, Kemb37.
High DM, poor healers: Bilagala, Kemb10, Polista.

2.2. Cultivar Experiment: comparison of root composition and wound-healing efficiency by cultivar

Roots of 15 cultivars from one trial and five cultivars from a second trial were air-freighted to the NRI in May 2000. Although three cultivars were common to both trials, they were considered separately, so that the trial was on 20 distinct lines (listed in Table 1). For each line, three roots were cubed and assessed for DM content (see below), and three further roots were cubed, samples freeze-dried and analysed for sugar content, (see below). For LS screening, toots were placed in controlled environment (CE) chambers maintained at 65% RH, 25 °C for 1 day. They were then wounded by removing a strip of periderm and left to heal for 5 days, after which the LS was assessed. The LS assessment was carried out in 4 experiments in each of which 10 lines were assessed, using 12 or 9 roots per line (arranged as 12 or 9 blocks across 3 CE chambers). Thus, for each line a total of 21 roots were assessed for LS.

2.3. Wounded Root Experiment: assessment of carbohydrate changes immediately under wounds during healing, and the relationship with LS

Roots were air-freighted to the NRI in May 2002. Twelve roots of each of ten cultivars (listed in Table 3) were placed in CE chambers maintained at 65% RH, 25 °C (arranged as 12 blocks across 3 CE chambers) for 1 day, after which they were wounded by removing a strip of periderm and then a thin section (approximately 20 mm × 40 mm, 4 mm thick) from one side of the root. This section, which had only a small amount of periderm remaining, was freeze-dried for subsequent sugar analysis. Wounded roots were maintained under the same conditions and left to heal for 5 days. The roots were weighed at the start of the experiment, before and after wounding and after 1, 4 and 5 days of healing. After 5 days, LS was measured for one half of the wound and a slice of surface tissue (approximately 4 mm thick) cut from the other half of the wound for freeze-drying and subsequent sugar analysis.

Table 1
Root composition and LS by cultivar in the Cultivar Experiment

Cultivar name	Mean LS	DM content	Sucrose (mg/g dry wt)	Fructose + glucose (mg/g dry wt)	Total sugars (mg/g dry wt)	Sucrose (mg/g water)	Fructose + glucose (mg/g water)	Total/sugars (mg/g water)
Yan Shu 1 (2)	0.94	24.0	57.7	125.0	184.3	18.2	39.5	58.2
Yan Shu 1	0.87	25.1	68.3	120.9	203.0	22.8	40.4	67.9
Blesbok	0.86	17.9	69.9	149.0	222.1	15.2	32.4	48.4
Santo Amaro	0.82	23.5	88.4	63.3	172.9	27.1	19.4	53.0
Zapallo (2)	0.80	18.7	98.3	124.6	226.3	22.6	28.7	52.1
Zapallo	0.78	19.8	164.9	71.5	244.6	40.7	17.7	60.4
Xu Shu 18	0.77	23.4	105.6	33.6	146.0	32.2	10.2	44.5
Brondal	0.71	18.6	151.8	60.1	215.5	34.7	13.7	49.2
Tainung No 64	0.64	21.7	93.9	102.5	198.1	26.1	28.5	55.0
Cemsa 74-228	0.62	25.5	127.8	41.8	174.0	43.6	14.3	59.4
Naveto	0.49	26.9	40.0	107.0	149.4	14.8	39.5	55.1
Kemb 10 (2)	0.46	30.6	99.4	2.7	106.8	43.8	1.2	47.1
Mafutha	0.39	25.9	132.9	23.1	172.2	46.5	8.1	60.2
Mugande	0.31	31.2	103.5	1.1	116.7	47.0	0.5	53.0
KSP 20 (2)	0.28	23.7	45.0	123.4	176.3	13.9	38.3	54.7
Kemb 37	0.27	23.3	30.4	103.6	141.0	9.2	31.4	42.7
NC 1560	0.26	20.2	90.5	112.9	211.9	22.8	28.5	53.5
Kemb 10	0.22	26.3	125.6	5.1	139.7	44.8	1.8	49.8
Mogamba	0.20	27.2	146.7	8.4	162.4	54.9	3.2	60.8
SPK 004 (2)	0.14	29.6	91.9	15.2	113.0	38.6	6.4	47.4

For each cultivar three roots were sampled and analysed for sugar composition and three different roots for DM. LS was assessed in four experiments in each of which 12 or 9 roots of each of 10 cultivars were assessed. Sugar levels in mg/g water were calculated as sugar (mg/g dry wt) × DM/(100−DM).
(2) indicates roots grown in a separate field trial. Although some of the cultivars were duplicated between the two trials, given the different growth conditions they were considered as separate lines for the purposes of this experiment.

2.4. Cuboid Experiment: assessment of carbohydrate changes in tissue cuboids during healing, and the relationship with LS

Roots were air-freighted to the NRI in September 2002. Three cuboids (5 mm × 15 mm × 50 mm) were cut from the central tissue (parenchyma) of each of 9 roots of each of 12 cultivars (listed in Table 4). All three cuboids were weighed. One was immediately frozen for subsequent freeze-drying and sugar analysis, while the other two were placed with the largest surface horizontal, on a plastic sheet in CE chambers maintained at 73% RH, 25 °C (arranged as 9 blocks across 3 CE chambers). After 1 day both remaining cuboids were weighed, one was frozen for freeze-drying and sugar analysis and the other returned to the CE chamber. This final cuboid was weighed after 4 and then 5 days, at which time it was cut into two. One half was weighed and frozen for freeze-drying and sugar analysis, while the other was assessed for LS. LS of whole roots of the cultivars used in this experiment were tested prior to the main experiment.

2.5. Assessment of lignification score

The method to assess LS was essentially as described in van Oirschot et al. (2006). Sections approximately 10 mm in depth were cut from the wounded area of whole roots. Three thin cross sections approximately 1 mm thick were cut from the wound, or in the case of the Cuboid Experiment, from the cuboid, using a razorblade. The sections were stained with phloroglucinol (1% in 95% ethanol) for 1 min, transferred to concentrated HCl for 1 min, then rinsed in water for 1 min. Each wound was given a score between 0 and 1 based on the continuity of lignification across the wound, and the average score was calculated. In the case of cuboids, the sections were cut across the whole tissue block, but the score related to lignification of the surface that had been uppermost in the incubator. In this case the scoring was carried out by two independent assessors and the mean value recorded. Analysis of the data using the two assessors as blocks indicated no significant difference between their assessments.

2.6. Dry matter content

DM content of whole roots was measured by oven drying (80 °C, 48 h) of 10 g samples selected randomly after the whole root had been diced into cubes of about 8 mm cubed. DM content of tissue near the wound (Wounded Root Experiment) and cuboids (Cuboid Experiment) were calculated using the sample weights before and after freeze-drying.

2.7. Sugar analyses

Freeze-dried samples were ground and extracted in water (1 g sample in 20 mLl water (Cultivar Experiment), or 200 mg in 4 mL water (Wounded Root and Cuboid experiments) by shaking for 1 h at room temperature. The extract was centrifuged (filtered for Cultivar Experiment), the supernatant diluted with acetonitrile to 80% acetonitrile and filtered through a 0.45 μm PTFE syringe filter. Twenty microlitre samples were injected onto an amino-bonded HPLC column (Hypersil APS-2, 20 cm), maintained at 30 °C, using 80% acetonitrile running at 0.8 mL/min as the mobile phase. Sugars were detected using a refractive index detector (Hewlett Packard), and peak areas were calculated using a PerkinElmer LCI-100 Integrator.

2.8. Experimental design and statistical analysis

For all experiments the roots or cuboids were arranged on shelves in 3 CE chambers in a randomised complete block design.

Statistical analyses were carried out using Genstat (Rothamsted, UK).

3. Results and discussion

3.1. Relationship between root composition and wound-healing efficiency

The Cultivar Experiment was conducted to examine the root composition for a range of sweetpotato cultivars and how these

Table 2
Relationship between LS measured at low humidity, DM content and sugar levels by cultivar for the Cultivar Experiment

	Correlation coefficient (r)	
	Lignification score	% Dry matter content
% Dry matter content	−0.535*	
Fructose + glucose (mg/g dry wt)	0.480*	−0.681**
Sucrose (mg/g dry wt)	n.s.	n.s.
Total sugars (mg/g dry wt)	0.592**	−0.882***
Fructose + glucose (mg/g water)	0.422+	−0.498*
Sucrose (mg/g water)	n.s.	0.489*
Total sugars (mg/g water)	n.s.	n.s.

The data used to calculate the correlation coefficients are those shown in Table 1.
$n = 20$.
n.s., not significant.
Symbols (+, *, **, ***) indicate significance to 10, 5, 1 and 0.1%, respectively.

related to wound-healing efficiency at low humidity, as indicated by LS. The data are summarised in Table 1 (cultivars ordered by decreasing LS), and relationships among characteristics are summarised in Table 2. As reported previously (van Oirschot et al., 2002, 2006) there was a wide range in LS, which correlated negatively with DM content. The main sugars in sweetpotato are sucrose, glucose, fructose and maltose. Generally maltose is low and fairly constant among cultivars and conditions. There was a very strong negative correlation between DM content and sugar (/DM). This is not unexpected, as an increase in DM (mostly starch) would result in a decrease in sugar/DM even if there were no change in sugar levels. Perhaps more interestingly there was a negative correlation between DM and monosaccharide (fructose and glucose) levels but not with sucrose levels. As expected there was also a positive correlation between LS and sugar. Again this appeared to be due to the levels of monosaccharides; and there was no relationship between LS and sucrose levels. We also calculated sugar levels in terms of water content, which gives an indication of overall sugar concentration in the cell. This calculation depends on several experimentally measured parameters and is therefore prone to greater error than sugar/DM. Monosaccharide levels/water, were positively correlated with LS, but this was not the case for sucrose levels/water. Interestingly, although monosaccharide levels/water were negatively correlated with DM, sucrose levels/water were positively correlated with DM.

3.2. Compositional changes close to the healing wound

The Wounded Root and Cuboid experiments were designed to enable us to look at compositional changes, in particular changes in sugar levels, in tissue close to healing wounds. In the Wounded Root Experiment, we assessed tissue composition at a wound made on an intact root both at the time of wounding, and after healing was complete (5 days). In the Cuboid Experiment, by considering the healing of tissue cuboids excised from the centre of the root, and using several cuboids cut from adjacent tissue from one root, we were able to assess sugar levels at wounding and after 1 and 5 days of healing. These two experiments also enabled us to compare the behaviour of individual roots, rather than just comparing cultivars, which provides a more powerful tool for investigating the role of physiological factors by using multiple regression analysis (see below).

The main data are summarised in Fig. 1 and Table 3 for the Wounded Root Experiment and Fig. 2 and Table 4 for the Cuboid Experiment. In all cases, the cultivars are ordered by decreasing LS. The relationship among characteristics are summarised in Tables 5 and 6.

Table 3
LS, weight loss and composition adjacent to wounds for roots from 10 sweetpotato cultivars (Wounded Root Experiment)

Cultivar	LS	Initial sugar level (mg/g dry wt)	Final sugar level under wound (mg/g dry wt)	Sugar increase (mg/g dry wt)	Initial sugar level (mmol/g dry wt)	Final sugar level under wound (mmol/g dry wt)	Sugar increase (mmol/g dry wt)	Initial DM%	Calculated water loss from under wound (% of initial tissue wt)
Cemsa	0.88	134.6	146.7	12.0	0.43	0.51	0.08	23.8	32.5
Zapallo	0.65	174.0	239.9	65.9	0.73	0.98	0.26	20.7	37.7
Sinia	0.53	121.8	129.4	7.6	0.37	0.44	0.06	29.7	42.0
Yanshu	0.48	152.2	155.5	3.3	0.56	0.59	0.03	21.2	45.5
KSP20	0.44	132.9	142.5	9.7	0.55	0.55	0.00	25.5	48.3
Kemb37	0.32	133.5	160.1	26.6	0.53	0.63	0.10	24.9	47.0
Kemb10	0.30	112.4	118.8	6.4	0.34	0.39	0.04	28.4	47.3
Polista	0.23	98.9	96.9	−2.0	0.30	0.33	0.02	32.2	44.9
Bilagala	0.18	107.7	118.0	10.3	0.37	0.47	0.11	27.3	44.9
Hernandez	0.10	194.6	194.0	−0.7	0.74	0.65	−0.09	22.2	52.9
Mean	0.41	136.2	150.1	13.9	0.49	0.55	0.61	25.6	44.2
Cult effect	***	***	***	***	***	***	***	***	***
LSD	0.24	22.6	25.6	26.5	0.10	0.12	0.11	2.1	6.7

12 roots were assessed for each cultivar. After removal of a section of periderm, a strip of tissue (3 mm thick) was removed to create a wound, and for analysis of initial composition (Sugar and DM). After 5 days LS was assessed, and a further strip of tissue (3 mm thick) removed for analysis.
Symbol (***) significant to 0.1.

Table 4
LS, weight loss and composition measured for tissue cuboids during healing, averaged by cultivar (Cuboid Experiment)

Cultivar name	LS	DM%	% wt loss (day 1)	% wt loss (day 4)	% wt loss (day 5)	Total sugars (day 0) mg/g dry wt	Total sugars (day 1) mg/g dry wt	Total sugars (day 5) mg/g dry wt	Sugar increase (day 1) mg/g dry wt	Sugar increase (day 5) mg/g dry wt	Total sugars (day 0) mmol/g dry wt	Total sugars (day 1) mmol/g dry wt	Total sugars (day 5) mmol/g dry wt
Zapallo	0.62	22.0	15.4	60.5	67.2	250.8	275.9	287.4	25.1	36.6	1.01	1.08	1.05
Cemsa-74	0.29	28.9	15.0	57.2	62.6	142.1	178.9	139.8	52.6	13.5	0.48	0.60	0.45
Kemb37	0.27	22.5	16.4	64.6	70.3	170.3	189.6	160.7	19.3	−9.5	0.81	0.85	0.65
KSP20	0.24	22.6	15.9	63.1	68.6	183.3	195.0	173.1	11.7	−10.2	0.88	0.88	0.70
Yanshu	0.22	31.6	15.5	56.9	61.5	130.8	169.1	130.3	34.1	14.0	0.46	0.58	0.43
B'regard	0.20	21.6	16.4	64.2	70.9	215.1	217.5	220.4	2.4	5.3	0.99	0.95	0.79
Bilagala	0.17	31.9	15.3	57.5	61.4	92.6	119.3	125.9	26.8	33.3	0.32	0.40	0.47
Naveto	0.14	28.4	15.2	60.3	64.9	135.1	168.4	137.7	33.3	2.6	0.63	0.73	0.50
Hernandez	0.12	21.0	17.6	68.0	73.4	213.7	228.7	227.7	15.0	14.1	0.98	0.98	0.81
Kemb10	0.12	31.8	16.5	57.7	61.4	113.0	152.9	143.5	39.9	30.5	0.35	0.47	0.49
Polista	0.12	37.6	15.3	51.3	54.4	85.1	116.8	102.1	31.7	17.1	0.26	0.36	0.34
Sinia	0.08	36.4	16.1	53.6	56.4	88.4	110.4	108.4	22.0	20.0	0.30	0.36	0.38
Mean	0.22	28.0	15.9	59.6	64.4	152.0	177.0	163.1	26.2	13.9	0.63	0.69	0.59
Cult sign. (p value)	<0.001	<0.001	n.s.	<0.001	<0.001	<0.001	<0.001	<0.001	n.s.	n.s.	***	***	***
LSD	0.13	2.3	2.0	3.3	3.2	38.4	41.6	45.9	37.7	45.8	0.18	0.19	0.18

Three cuboids were cut from the central tissue of each of 9 roots of each of 12 cultivars. Cuboids were sampled and analysed after 0, 1 and 5 days. Cuboids were placed to wound-heal with the largest surface horizontal, on a plastic sheet in controlled environment chambers maintained at 73% RH, 25 °C. Cuboids were placed to wound-heal with the largest surface horizontal, on a plastic sheet in controlled environment chambers maintained at 73% RH, 25 °C.

Fig. 1. Sugar levels near wounds of roots of ten sweetpotato cultivars at the time of wounding and after 5 days of healing (Wounded Root Experiment). Twelve roots were assessed for each cultivar. After removal of a section of periderm, a strip of tissue (3 mm thick) was removed to create a wound, and for analysis of initial sugar levels. After 5 days of healing at 65% RH, 25 °C a further strip of tissue (3 mm thick) removed for repeat analysis. Error bars indicate S.E. for total sugars.

Fig. 2. Sugar content of root tissue cuboids of 12 sweetpotato cultivars at day 0, 1 and 5 of healing in the Cuboid Experiment). Three cuboids were cut from the central tissue of each of 9 roots of each of 12 cultivars. They were placed to wound-heal with the largest surface horizontal, on a plastic sheet in controlled environment chambers maintained at 73% RH, 25 °C. Error bars indicate S.E. for total sugars.

As previously observed there is a wide range among cultivars in ability to wound-heal (LS) for both experiments. In the Cuboid Experiment the extent of lignification was generally lower (LS ranging from 0.08 to 0.62) than for the Wounded Root Experiment (LS ranging from 0.10 to 0.88) and was also lower than we have found in previous experiments on whole roots. Nevertheless, a correlation of wound-healing efficiency by cultivar of cuboids compared to whole roots ($r = 0.623$, significant at 5%) indicates that the relative performance of cultivars was reasonably consistent in the two systems.

Table 5
Relationship (correlation coefficient, r) by individual root between wound-healing score (LS) and other root factors (Wounded Root Experiment)

	LS	DM (day 0)
DM (day 0)	−0.270**	
Total sugar content (day 0)	n.s.	−0.630***
(mmol/g DM)	n.s.	
Monosaccharide content (day 0)	n.s.	−0.553***
Total sugar content (day 5)	0.246*	−0.610***
(mmol/g DM)	0.202*	
Monosaccharide content (day 5)	n.s.	−0.409***
Sugar increase (day 5)	0.302**	−0.165+
(mmol/g DM)	0.292**	
Calculated water loss from under wound	−0.399***	n.s.

120 roots, 12 from 10 cultivars were assessed during wound-healing as described in the legend to Table 3.
Sugar levels are calculated as mg/g DM unless otherwise stated.
Symbols (+,*,**, ***) significant to 10, 5, 1 and 0.1%, respectively; n.s., not significant.

Table 6
Relationship (correlationship coefficient, r) by individual root between wound-healing score (LS) and other tissue characteristics, measured in the Cuboid Experiment)

	LS	DM (day 0)
DM (day 0)	−0.410***	
Total sugar content (day 0)	0.456***	−0.838***
(mmol/g DM)	0.389***	
Total sugar content (day 1)	0.579***	−0.751***
(mmol/g DM)	0.525***	
Total sugar content (day 5)	0.399***	−0.695***
(mmol/g DM)	0.424***	
Sugar increase (day 1)	0.244*	0.199 *
(mmol/g DM)	0.301**	
% wt loss (day 1)	−0.161 n.s.	−0.113 n.s.
% wt loss (day 4)	−0.040 n.s.	−0.756 ***
% wt loss (day 5)	0.156 n.s.	−0.898 ***

Three cuboids were cut from the central tissue of each of 9 roots of each of 12 cultivars. as described in the legend to Table 4.
Sugar levels are calculated as mg/g DM unless otherwise stated.
Symbols (+,*,**, ***) significant to 10, 5, 1 and 0.1%, respectively; n.s., not significant.

For both experiments, as observed previously, there was a negative correlation between LS and DM content, although there are outstanding cultivars, notably Hernandez, which has a relatively low DM content (22.2, 21.0% in the two experiments) but is a poor healer in both cases. As observed before, there is also a strong negative correlation between DM content and sugar/DM.

In the Wounded Root Experiment calculation of water loss from under the wound (using measurements of DM) indicated that water loss was less where LS was high (correlation $r = 0.399$ $p < 0.001$), which supports the validity of the LS assessment for assessing wound-healing.

In the case of the Wounded Root Experiment in contrast to the Cultivar Experiment no significant relationship was observed between sugar levels before wounding and LS. It is possible that this is a result of examining surface cortex tissues in the Wounded Root Experiment, compared to the whole root sugar levels of the Cultivar Experiment. The main sugars found in sweetpotato are sucrose, glucose, fructose and maltose. Generally sucrose is the main sugar, and the levels of glucose and fructose are lower and approximately equal. Sugar levels increased close to the wound over the 5 days of healing. The pattern of increase was rather variable among cultivars; although the greatest increase was usually for glucose and fructose, for some cultivars the main increase was in sucrose. The better wound-healers tended to exhibit a greater rise in sugar levels, resulting in a significant positive correlation between LS and both total sugar levels at day 5, and the rise in sugars (Table 5). In this experiment no relationship was found specifically between LS and monosaccharide levels either before or after healing.

In the case of the Cuboid Experiment, as for the Wounded Root Experiment there was also an accumulation of sugars during the healing period (Fig. 2) although it can also be seen that there was a greater accumulation of sugars over the first day from the time the cuboids were cut, and in most cases a subsequent decline from day 1 to day 5. The cultivar sugar levels for day 0 and day 5 data are very consistent between the Wounded Root and Cuboid experiments (Correlation of total sugar levels $r = 0.846$ (significant at 1%), 0.933 (significant at 0.1%) for day 0 and 5, respectively). We assume, although we could not do the measurements, that there was an initial accumulation beneath the wounds of the intact roots as well, so that the maximum sugar levels would have been higher than those measured on day 5. However, it is possible that sugar accumulation in whole roots involves some translocation, which cannot occur in the cuboids. It is notable that in the cuboids, the increase in sugar levels was almost entirely due to an increase in sucrose levels. Unlike the Wounded Root Experiment, there was a positive correlation between LS and initial sugar levels. This may be because cuboids were cut from the central parenchyma tissue whereas in the Wounded Root Experiment the wound was confined to the surface cortex, although there is also a possibility that some breakdown of starch to sugars occurred in the cuboids during cutting and weighing before they were frozen in liquid nitrogen. The correlation was stronger with sugar levels at day 1. In neither experiment was there a significant correlation between monosaccharide levels and LS at any timepoint, although a trend for a positive relationship was observed.

Table 7
Linear regression models of LS in terms of root characteristics (Wounded Root Experiment)

Variance accounted for (adjusted R^2) (%)	Model
29.8	0.175 + cultivar
6	0.999 − 0.023 DM
34	1.138 − 0.035 DM + cultivar
9.4	0.368 + 0.00316 sugar increase
35.8	0.149 + 0.00256 sugar increase + cultivar

120 roots, 12 from 10 cultivars were considered in these models. DM is expressed as % and sugar increase as mg/g DM.

3.3. Linear regression models of LS

As a tool for examining the role of physiological factors in controlling wound-healing efficiency, linear regression models of root and cuboid LS in terms of both the measured characteristics and of cultivar were constructed (Tables 7 and 8). The strong relationship between DM content and sugar levels, means that the two cannot be included in the same models. LS can be modelled in terms of DM content. However, better models (i.e. accounting for a higher % variance) can be obtained by considering sugar increase for the Wounded Root Experiment, and sugar levels after day 1 for the Cuboid Experiment. Notably, a model using sugar levels after 1 day accounted for 33% variance among cuboids. This relationship is illustrated in Fig. 3. The observation that sugar level/increase account for more % variance than models using cultivar alone, indicates that sugar level/increase is a direct contributing factor. For all models, the addition of cultivar as a factor increased the % variance accounted for, suggesting that other cultivar factors in addition to sugar level/increase are important.

In summary, comparison of the levels of variance accounted for by the models indicates that LS is strongly cultivar dependent, and most of the cultivar effect is related to cultivar differences in sugar levels during wound-healing. Differences in sugar levels between roots/cuboids of each cultivar also have an effect. Further, the similarity of the models for the Wounded Root and Cuboid experiments

Table 8
Linear regression models of LS in terms of root tissue characteristics (Cuboid Experiment)

Variance accounted for (adjusted R^2) (%)	Model
38	0.200 + cultivar
20	−0.0008 + 0.0014 sugars_day 0
40.9	0.014 + 0.0086 sugars_day 0 + cultivar
32.9	−0.106 + 0.0018 (sugars _day 1)
50.1	−0.136 + 0.0015 (sugars_day 1) + cultivar
15.4	0.021 + 0.0012 (sugars_day 5)
37.7	0.116 + 0.00038 sugars_day 5 + cultivar
16.1	0.597 − 0.0136 DM
45.9	0.719 − 0.024 DM + cultivar

Cuboids from 9 roots of each of 12 cultivars were considered in these models. DM is expressed as % and sugar increase as mg/g DM.

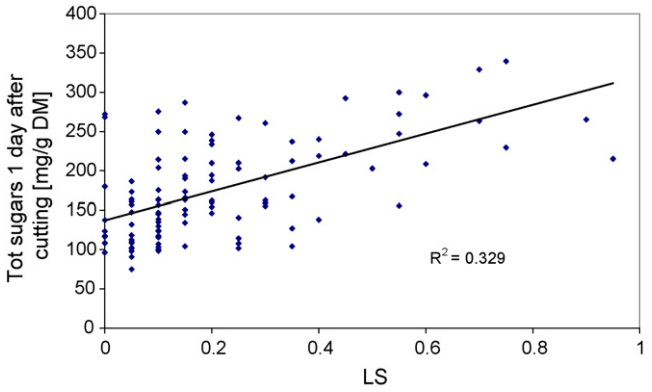

Fig. 3. Relationship between LS and total sugar content 1 day after cuboids were cut in the Cuboid Experiment. Three cuboids were cut from the central tissue of each of 9 roots of each of 12 cultivars. Cuboids were placed to wound-heal with the largest surface horizontal, on a plastic sheet in controlled environment chambers maintained at 73% RH, 25 °C. One of the three cuboids was sampled for sugar analysis at day 1, while another was used to assess LS at day 5. Each data point corresponds to a separate root (n = 108).

gives us confidence in the validity of using the cuboids to investigate wound-healing of whole roots. In the Cuboid Experiment we chose for convenience to measure sugar levels after 1 day of healing. It is probable that maximum levels would have occurred either earlier or later, and that peak sugar levels would provide an even better model of wound-healing efficiency. We might therefore have underestimated the contribution of this mechanism to wound-healing efficiency.

3.4. Osmotic protection, DM content and the susceptibility of tissue to water loss

Our findings suggest that mobilisation of sugars helps to protect the tissues and allow wound-healing in the presence of water stress, although the mechanism by which this occurs is less clear. Several studies have shown that in cases of water stress, plant tissues may accumulate sugars. For example, Quick et al. (1989) showed that leaf discs stressed by being floated on high concentrations of mannitol accumulated sucrose. Geigenberger et al. (1997, 1999) obtained similar results when they studied water stress in potato tubers by imposing osmotic stress on tissue discs. In both systems an increase in sucrose was attributed to the activation of sucrose phosphate synthase (SPS). Under more severe water stress, Geigenberger et al. (1999) postulated that there was also an inhibition of starch synthesis and breakdown of starch. They suggested that sucrose cycling occurs, as part of the control of the sucrose:starch ratio and is an important mechanism to protect against water stress. Wang et al. (2000) studied normal and osmotically stressed (0.6 M sorbitol) cultured sweetpotato cells. Stress led to an increase in sucrose and SPS activity. Northern blot analysis indicated an increase in SPS gene expression. Stress also led to an increase in sucrose cleaving enzymes alkaline invertase and sucrose synthase. In that study labelling experiments supported cycling of sucrose.

The precise physiological role of sucrose accumulation in these cases, as for sugar accumulation in our system, is not clear. One hypothesis is that the increase in osmotic potential could help to prevent water loss, although as we do not fully understand the compartmentalisation of osmolytes, it is very difficult to calculate whether the increase in sucrose levels is sufficient for this (Hare et al., 1998). For our system, in order to examine the possible role of sugars in osmotic regulation it is more relevant to consider sugar levels in terms of molar concentration rather than by weight. Thus, total sugars in terms of mmol/g dry weight are also given in Tables 3 and 4, and correlations with other characteristics in Tables 5 and 6. The trends in the data that we noted above remain the same.

If osmotic protection of overall root tissue is occurring, we would expect to see some relationship between sugar levels and water loss at the start of the healing process. In whole root experiments it has been very difficult to compare initial rates of water loss between cultivars due to differences in root size and shape, and non-uniformity of wound size. The use of tissue cuboids could remove these problems. However, in our experiments staining indicated that effective healing did not occur on the lower surface of the cuboids, probably due to limited access of oxygen to the lower surface which was pressed directly against a plastic surface. This meant that rate of weight loss could not be used as an indication of water loss from the wound. In fact in the Cuboid Experiment no significant difference among cultivars in rate of weight loss for day 1 was observed (Table 4).

There is evidence that in other root crops high dry matter content is associated with a less efficient response to wounding. In cassava, postharvest physiological deterioration (PPD) is an enzymatically driven response that occurs throughout the root as a result of wounding and oxidative stress incurred at harvest, and severely limits the root shelf-life (Beeching et al., 1999). It is known that PPD is less when roots are placed at high humidity immediately after harvest, implying that wound-healing reduces the symptoms. Further it has been observed that PPD is less where the sugar:starch ratio is high, for example, in certain cultivars and after pre-harvest pruning (van Oirschot et al., 2000). This is consistent with the behaviour of sweetpotatoes and suggests that PPD is reduced where the root is able to facilitate wound-healing by accumulation of sugars.

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